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Functional expression of eukaryotic membrane proteins in *Lactococcus lactis*

Monné, Magnus; Chan, Ka Wai; Slotboom, Dirk-Jan; Kunji, Edmund R.S.

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Supplementary Material

Functional expression of eukaryotic membrane proteins in *Lactococcus lactis*

M. Monné, Ka Wai Chan, Dirk Jan Slotblom, and Edmund R. S. Kunji

Protein Science **14**(12) 2005

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::* ::*****:*** *:*****:*
AAC1 1 MSHTEQTQSSHFGVDFLMGGVSAAIAKTGAAPIERVKLL 40
AAC2 1 MSSNAQVKTPLPAPAPKKESNFLIDFLMGGVSAAVAKTAASPIERVKLL 50
AAC3 1 MSSDAKQQETNFAINFLMGGVSAAIAKTAASPIERVKIL 39

:***:***:***:*** :* **:***** :***:*****:*****
AAC1 41 MQNQEEMLKQGSLDTRYKGIIDCFKRTATHEGIVSFWRGNTANVIRYFPT 90
AAC2 51 IQNQDEMLKQGTLDTRYKGIIDCFKRTATQEGVISFWRGNTANVIRYFPT 100
AAC3 40 IQNQDEMIKQGTLDKKYSGIVDCFKRTAKQEGVISFWRGNTANVIRYFPT 89

***** : : :* :** ***** ***** *****
AAC1 91 QALNFAFKDKIKSLLSYDRERDGYAKWFAAGNLFSGGAAGGLSLLFVYSLD 140
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AAC3 90 QALNFAFKDKIKLMFGFKKE-EGYKWFAGNLFSGGAAGALSLLFVYSLD 138

:*****: : ** ***** *****:***: *****:***:*
AAC1 141 YARTRLAADARGSKSTSQRQFNGLLDVYKKTLLKTDGLLGLYRGFVPSVLG 190
AAC2 150 YARTRLAADSKSSKKGARQFNGLIDVYKKTLLKSDGVAGLYRGFLPSVVG 199
AAC3 139 FARTRLAADAKSSKKGARQFNGLTDVYKKTLLKSDGIAGLYRGFMPSVVG 188

*:*****:***:***:***:***:***:*****:*** *****
AAC1 191 IIVYRGLYFGLYDSFKPVLLTGALEGSFVASFLLGWVITMGASTASYPLD 240
AAC2 200 IIVYRGLYFGMYDSLKPLLLTGSLEGSFLASFLGWVVTGASTCSYPLD 249
AAC3 189 IIVYRGLYFGMFDLKLPLVLTGSLDGSFLASFLGWVVTGASTCSYPLD 238

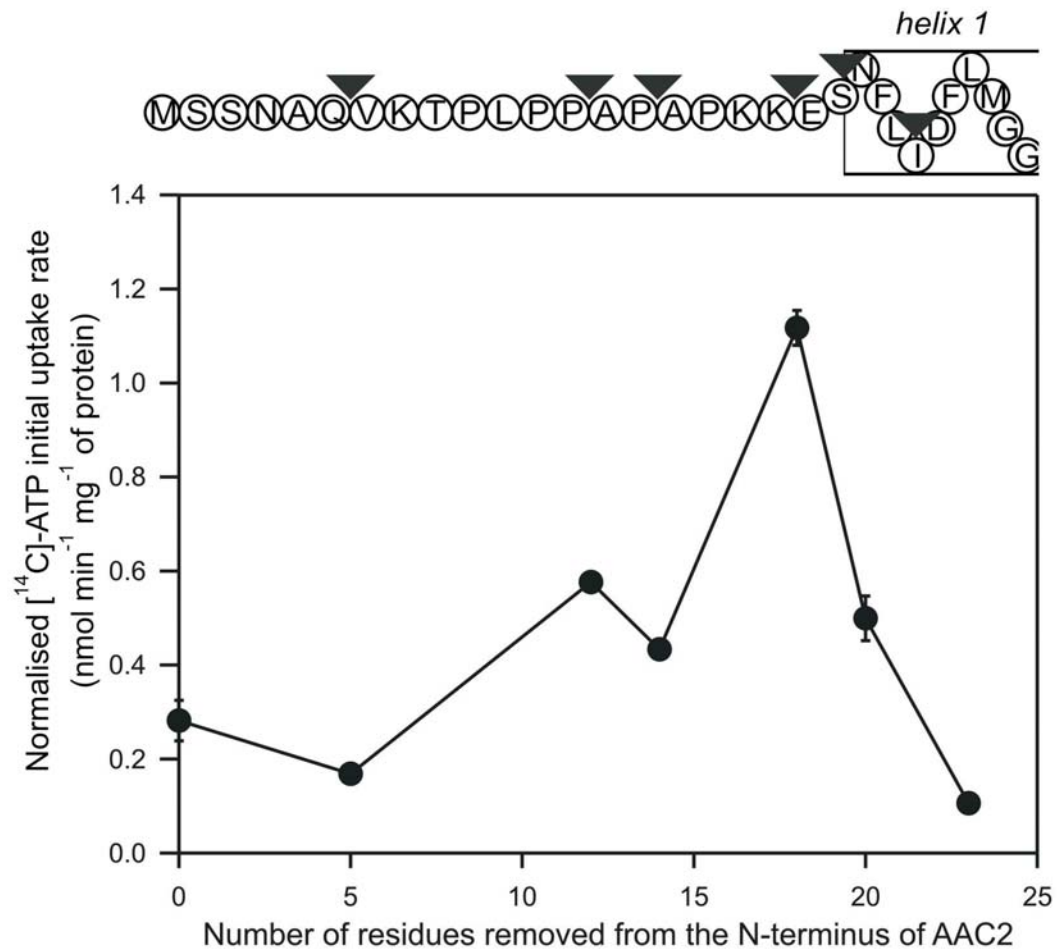
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AAC2 250 TVRRRMMMTSGQAVKYDGAIDCLRKIVAAEGVGSFLFKGCGANILRGVAGA 299
AAC3 239 TVRRRMMMTSGQAVKYNGAIDCLKKIVASEGVGSFLFKGCGANILRGVAGA 288

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AAC3 289 GVISMYDQLQMILFGKKFK 307

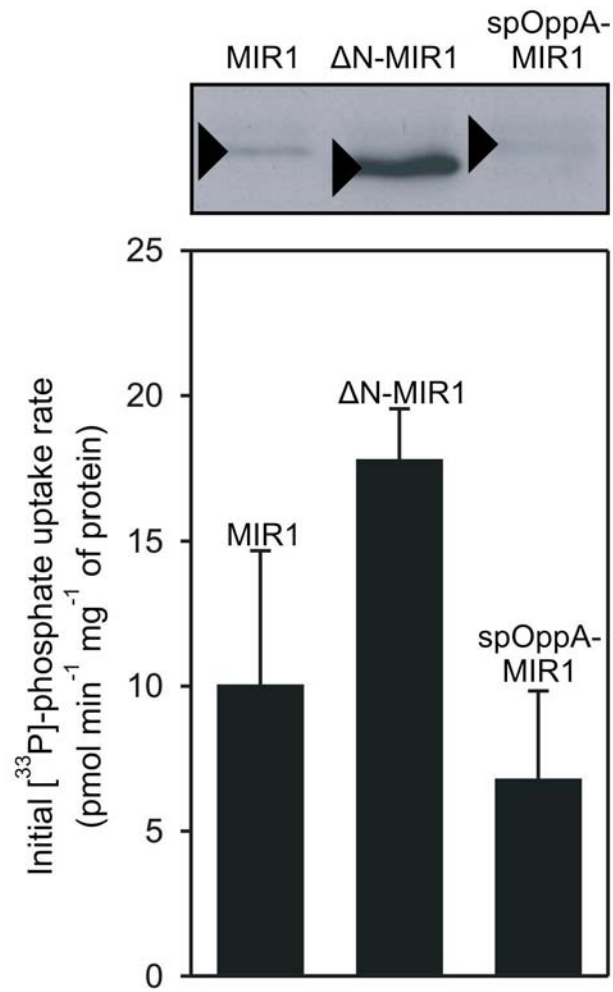
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Supplementary Figure 1. Alignment of the amino acid sequences of the three ADP/ATP carriers from *Saccharomyces cerevisiae* AAC1, AAC2 and AAC3.

Identical residues are indicated by an asterisk and the similar amino acids by a colon.

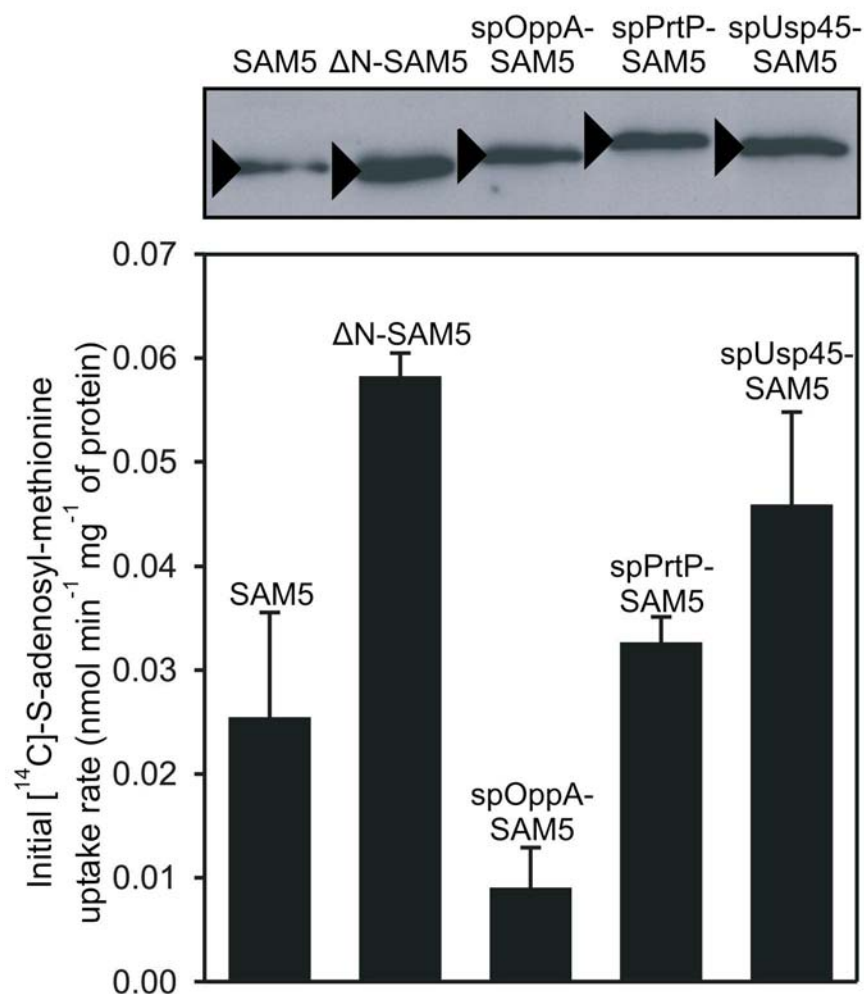


Supplementary figure 2. The normalised specific activity of ADP/ATP carrier AAC2 and the amino-terminal truncation mutants. Schematic representation of the N-terminal region of AAC2 (top) and the normalised initial uptake rate (bottom) for each of the truncation mutants (indicated by arrow heads). The normalised initial uptake rates is expressed as the initial uptake rate of the truncation mutants corrected for the expression levels, normalised to the one of AAC2. The data are taken from Figure 4 Monné *et al.*



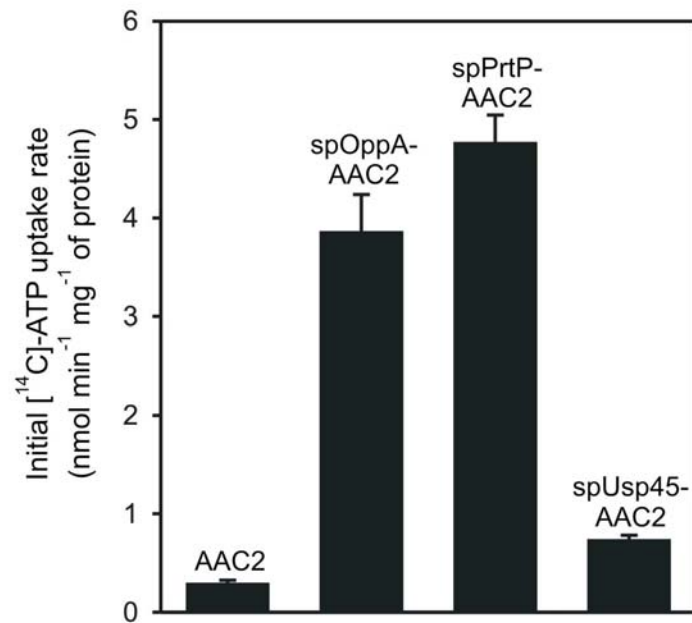
Supplementary Figure 3. Expression and transport activity of the different versions of yeast phosphate carrier MIR1

(a) Western blot (top) and the initial phosphate uptake rates (bottom) for the wild type MIR1, truncation mutant ΔN-MIR1 and spOppA-MIR1 fusion. The initial transport rates were corrected for the background. The initial uptake rates, measured after 4 min, are the average of three independent experiments carried out in triplicate with the standard error of the mean (SEM).



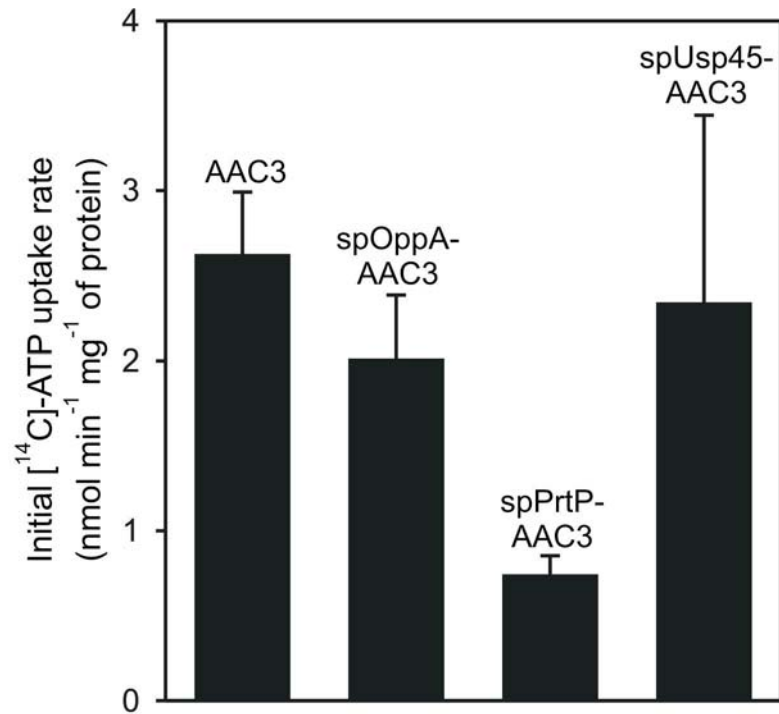
Supplementary Figure 4. Expression and transport activity of the different versions of yeast S-adenosyl-methionine carrier SAM5

(a) Western blot (top) and the initial S-adenosyl-methionine uptake rates (bottom) for the wild type SAM5, truncation mutant ΔN-SAM5 and spOppA-SAM5, spPrtP-SAM5 and spUsp45-SAM5 fusions. The initial transport rates were corrected for the background. The initial uptake rates, measured after 8 min, are the average of three independent experiments carried out in triplicate with the standard error of the mean (SEM).



Supplementary Figure 5. Transport activity of the different versions of yeast ADP/ATP carrier AAC2

The initial ATP uptake rates for the wild type AAC2 and the spOppA-AAC2, spPrtP-AAC2 and spUsp45-AAC2 fusions. The initial transport rates were corrected for the background. The initial uptake rates, measured after 15 s, are the average of three independent experiments carried out in triplicate with the standard error of the mean (SEM).



Supplementary Figure 6. Transport activity of the different versions of yeast ADP/ATP carrier AAC3

The initial ATP uptake rates for the wild type AAC3, truncation mutant $\Delta\text{N-AAC3}$, and the spOppA-AAC3, spPrtP-AAC3 and spUsp45-AAC3 fusions. The initial transport rates were corrected for the background. The initial uptake rates, measured after 15 s, are the average of three independent experiments carried out in triplicate with the standard error of the mean (SEM).